

Attraction of *Cacopsylla pyricola* (Hemiptera: Psyllidae) to Female Psylla in Pear Orchards

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ABSTRACT The pear psylla, *Cacopsylla pyricola* (Förster), is a major economic pest of pears in North America and Europe. Laboratory studies have shown that males of both the summerform and winterform morphotypes in this species are attracted to volatiles given off by females. This study tested whether attraction by males to females can be shown in the field. We showed that males had a clear preference for sticky traps that had been baited with live females compared with traps baited with live males or traps that were left unbaited. Female *C. pyricola* did not exhibit a preference among the three types of trap treatments. These results were obtained for both morphotypes. Trap catch was also monitored at 2-h intervals to assess whether capture of males on female-baited traps varied with time of day. Summerform male *C. pyricola* were caught in highest numbers between 1445 and 1645 hours, whereas winterform males were most often captured between 1300 and 1700 hours on traps baited with females. In both trials, there was again a significant preference by males for the female-baited traps compared with unbaited traps. Long-term practical benefits of the methods developed here provide a platform for the development of more effective monitoring tools, use in mating disruption, or development of lure and kill technologies.

KEY WORDS *Cacopsylla pyricola*, pear psylla, diel periodicity, sex attraction

The pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), is a major economic pest of pears across North America and Europe. The primary economic damage caused by *C. pyricola* is fruit russetting, which results from honeydew on the fruit that is produced by the psylla nymphs. Pear psylla is a multivoltine, seasonally dimorphic species that may have as many as five generations per year (Horton 1999). The seasonal dimorphism is controlled by photoperiod: the summerform morphotype is present during the longer photoperiods of late spring through early autumn, whereas the overwintering form (winterform morphotype) is present between mid-autumn and early spring (Oldfield 1970). Winterform psylla overwinter in reproductive diapause, which ends in late December (Krysan and Higbee 1990). Mating in winterforms is delayed until mid-February when temperatures begin to rise (Krysan and Higbee 1990). The first summerforms appear in the orchards beginning in early May (Oldfield 1970). Both sexes of the summerform morphotype are reproductive within a few days of eclosion (Burts and Fischer 1967).

Our knowledge of mating behavior and the roles that different cues may play in mate-locating behavior by *C. pyricola* and related psyllids is notably incomplete. It is known that a number of psyllid species use

acoustic cues in locating or accepting mates (Tishechkin 1989, 2006; Percy et al. 2006), although this behavior has yet to be shown in *C. pyricola*. Recent evidence shows that males in some psyllid species use volatile chemicals to locate mates. Soroker et al. (2004), using a Y-tube olfactometer, showed that volatile chemicals mediated intersexual communication in the closely related pear psylla, *Cacopsylla bidens* (Šulc). Electroantennogram assays further showed a positive male response to females and to volatiles collected from females (Soroker et al. 2004). More recently, Horton and Landolt (2007) used choice-test and Y-tube olfactometer assays to show that *C. pyricola* males are attracted to volatile chemicals emitted by females. A study by Horton et al. (2007) used olfactometer assays to determine how female attractiveness in winterforms changed seasonally with diapause status.

All previous studies that have examined sex attraction in *C. pyricola* have been done in the laboratory, with no information yet available about mate-seeking activities in the field. Horton (1993) used sticky traps to monitor movement by male and female pear psylla of both morphotypes in pear orchards and showed that sticky trap catch during the reproductive generations was often highly male biased. However, that study did not discriminate between trap capture associated with mate-seeking behavior by males and trap capture caused by other activities such as search for feeding sites. Thus, there currently is no information about the role of mate-seeking behavior in affecting movement

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Fig. 1. (A) Inner organdy bag of psylla trap containing the attractant (live females, live males, or unbaited). (B) Completed trap composed of external screen and inner organdy bag. (Online figure in color.)

by the pear psylla in the field. Here, we tested whether female *C. pyricola* attracted male pear psylla in the field. Experiments were conducted for both the summerform and winterform morphotypes. We also report on the diel pattern of attraction in both morphotypes.

Materials and Methods

Trap Design. Sticky traps were used to assess whether limbs baited with male or female pear psylla attracted conspecific psylla in the field. Traps consisted of an inner cage that held the attractant (live female or male psylla) and an outer screen designed to capture psylla that came into contact with the trap. The outer screen was composed of a section of light brown-colored nylon with a 1 by 1-mm mesh (Fig. 1B). The inner cage consisted of an organdy bag (20 by 30 by 15 [at the widest point] cm in size) of a much finer mesh that held live male or female psylla. Each organdy bag was placed over a pear limb (Fig. 1A) and securely tied with a twist tie once the appropriate number and sex of psylla had been added (see below for specific numbers of insects). Limbs were first cleaned of resident psylla by sharply rapping the limb with a section of rubber hose; as necessary, psylla and other insects not dislodged with this method were removed manually. The nylon section was wrapped around the organdy bag, stapled along its margins to form an envelope, and coated with a thin layer of Tanglefoot Olson Sticky Stuff (Tanglefoot, Grand Rapids, MI). Each surrounding nylon envelope was ≈ 30 by 20 by 15 cm in size (Fig. 1B). This same trap design was used in all tests described below.

Summerform Trial. The first trial with summerform psylla was done in the summer of 2006 at an organic commercial pear orchard located near Zillah, Yakima Co., WA. Pear cultivars were Anjou and Bartlett. Trees

were ≈ 4.5 m in height, with 3-m spacing between trees. Adult male and female summerforms were collected on a 72 by 72-cm beat tray by rapping pear branches with a 60-cm length of rubber hose. Adults were visually separated by sex and aspirated into vials on the morning of the experiment. The sex of adults was determined by examining the posterior end of the abdomen. Males are readily identified by the presence of parameres and a proctiger that protrude dorsally; in contrast, the terminal end of the female's abdomen tapers smoothly downward to a point (Hodkinson and White 1979). Sixteen adults of one sex were placed into each of twenty 30-ml plastic vials, with 10 vials per sex per sampling period. The vials were kept in a cooler ($\approx 5^{\circ}\text{C}$) until the psylla were placed in the traps.

The experiment was set up in a randomized complete block design consisting of 20 blocks. Within each block, a single replicate of each of three treatments was deployed: traps baited with 16 live females, traps baited with 16 live males, or traps left unbaited (control). The treatments were placed at 1.5–2 m in height, at a density of one trap per tree on three similarly sized adjacent trees. Treatments within a block faced in the same cardinal direction. A minimum of two trees (10–12 m) separated adjacent blocks. Traps were left in the field for 4 d.

Blocks 1–10 were monitored 17–21 July 2006, whereas blocks 11–20 were assayed 28 July to 1 August 2006. High temperatures ranged from 28.2 to 38.1 $^{\circ}\text{C}$ (17–21 July) and 21.9 to 28.6 $^{\circ}\text{C}$ (28 July to 1 August). Low temperatures ranged from 11.3 to 19.2 $^{\circ}\text{C}$ (17–21 July) and 8.2 to 14.1 $^{\circ}\text{C}$ (28 July to 1 August). The relative humidity averaged 52% from 17 to 21 July and 54% for 28 July to 1 August. Skies were mostly sunny throughout the studies. On termination of the experiments (i.e., after 4 d in the field), the traps were collected and wrapped with wax paper for transfer

back to the laboratory. Numbers of male and female psylla on each trap were determined using a $\times 10$ hand lens.

Effects of treatment (branch baited with females, branch baited with males, or branch left unbaited) on numbers of male or female psylla per trap were assessed using analysis of variance (ANOVA). Separate statistical analyses were done for counts of trapped male and female psylla. The count data were square root transformed before analysis (Sokal and Rohlf 2001). The data were analyzed as a 3 by 2 (treatment by sampling interval) factorial design, with block nested within sampling interval (Gomez and Gomez 1984). The analyses were done by ANOVA using PROC MIXED in SAS (SAS Institute 2003). In the event of a significant treatment by sampling interval interaction, the SLICE command in PROC MIXED was used to examine treatment effects for each sampling interval separately. Specific treatment comparisons of interest were made using least significant difference (LSD) tests.

Winterform Trial. The winterform field experiment was done in two pear orchards between 1–7 and 13–19 March 2007. One set of traps was placed in a conventionally managed orchard near Wapato, Yakima Co., WA. The dominant pear cultivar was Bartlett. Average height of trees was ≈ 4 m, with 2.8-m spacing between trees. The second set of traps was placed in an orchard at the U.S. Department of Agriculture's Moxee Experimental Farm located 24.2 km east of Moxee, Yakima Co., WA. This orchard contains both Bartlett and Anjou cultivars but only Bartlett trees were used as they best approximated the size and spacing of the trees used in the other orchard. Two orchards were used because the grower at the Wapato site began his spring insecticide program immediately after the 1–7 March trial and before the second set of traps (13–19 March) could be set out. The psylla to be used in baiting the traps were collected the day before the start of each trial using the same techniques as were used in the summerform tests described above. Sixteen adults of each sex were sorted into separate 30-ml plastic vials for use in baiting the traps.

As in the summerform psylla trial, the winterform psylla experiment was set up in a randomized complete block design with 10 blocks at each of two orchards. Each block consisted of the same three treatments used in the summerform trial. The traps were set up using the same techniques that were used in the summerform psylla field experiment described above except that the traps were left out for 6 rather than 4 d. Blocks 1–10 (Wapato site) were monitored 1–7 March 2007, whereas blocks 11–20 (Moxee site) were monitored 13–19 March 2007. High temperatures ranged from 1.6 to 6.9 (1–7 March) and 9.9–21.2°C (13–19 March), whereas low temperatures ranged from –4.3 to 2.8 (1–7 March) and –5.2 to 3.8°C (13–19 March). The relative humidity averaged 88% from 1 to 7 March and 68% from 13 to 19 March. The weather varied from mostly sunny to trace amounts of snow during the experiment. When taking down the experiment at each site, the traps were collected and treated as in the

summer experiment. The data were analyzed similarly to the summerform data, except that block was nested within orchard (Gomez and Gomez 1984).

Summerform Diel Periodicity. Diel periodicity in numbers of pear psylla captured on traps was assessed in June 2007 at the Moxee Experimental farm. Adult female *C. pyricola* were collected from pear trees in the early afternoon on the day the experiment was set up. The psylla were placed into twenty 30-ml plastic vials each containing ~ 16 females. The study was set up in a randomized complete block design with 20 blocks and two treatments: control traps (no insects) and traps baited with 16 females (we eliminated the male-baited traps from this trial, because the previous tests indicated that this treatment had no effect on trap catch). On the evening preceding the start of the experiment, all of the traps were cleared of *C. pyricola* using forceps. The traps were checked over the following 3 d at every 2 h between 0645 and 2045 hours. The photoperiod was from 0430 to 2045. Each 0645 count included all psylla that were caught between 2045 (of the preceding day) and 0645 hours. As each trap was examined, psylla and all other trapped insects were removed from the traps using forceps. High temperatures ranged from 31.5 to 37.4°C, whereas low temperatures varied between 2.5 and 9.8°C over the 3-d experiment; the average temperature was 24.1°C. Average relative humidity during the study was 32.2%.

The effect of treatment (traps baited with females versus unbaited traps) on numbers of psylla per trap over each 2-h time block was assessed using a repeated-measures ANOVA. Time of day was the repeated-measures factor. Data for male and female captures were analyzed separately. The count data for each trap, at each time interval, were averaged over the 3 d within each time interval and the means were transformed as the natural log ($x + 1$) (Sokal and Rohlf 2001); a square-root transformation (as used in the previous analyses) failed to normalize the data. Profile contrasts were extracted to compare trap catch between adjacent time intervals, allowing us to determine statistically the time of day at which trap catch peaked. Statistical analyses were done using PROC MIXED in SAS (SAS Institute 2003).

Winterform Diel Periodicity. Diel periodicity in numbers of winterform psylla caught on traps was assessed for 3 consecutive d in early March 2008 at the Moxee Experimental farm. Adult female *C. pyricola* were collected from pear trees midday on the day the experiment was set up. The psylla were collected and treated the same as in the summerform pear psylla diel experiment above. The winterform experiment was designed and monitored the same as in the summerform study, with the only exception being the times the traps were checked. Because of the shorter photoperiod (0630–1730 hours) in early March compared with the June summerform psylla trial, the number of psylla caught on traps was assessed six times per day rather than eight times per day as done in the summerform trial. The first count was done at 0700 hours; the final count was taken at 1700 hours. The 0700 count included psylla trapped between 1700 the previous

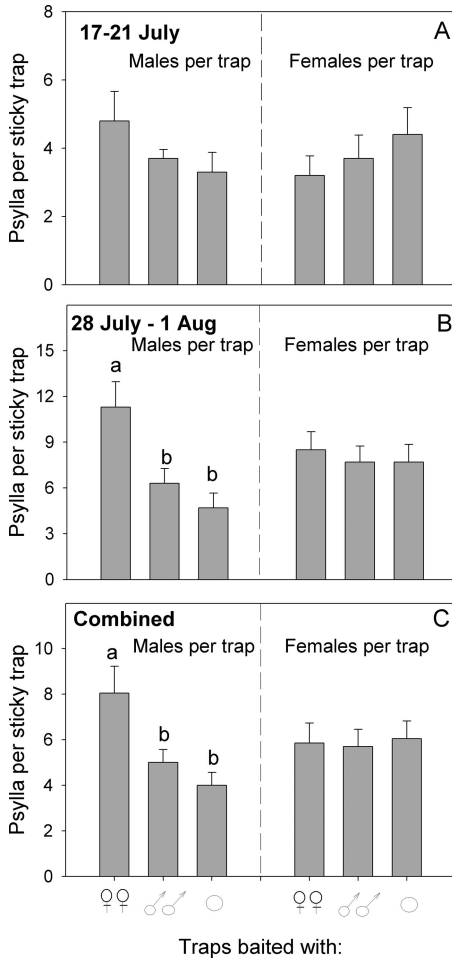


Fig. 2. (A) Mean (\pm SEM) numbers of summerform psylla caught per trap for each treatment during the 17–21 July 2006 trapping interval. (B) Mean (\pm SEM) numbers summerform psylla caught during the 28 July to 1 August trapping interval. (C) The combined means (\pm SEM) from dates 1 and 2. Double male symbols on the x-axis represent traps baited with male psylla, female symbols represent traps baited with females, and the open circles indicate results for the unbaited traps. Means with different letters are significantly different (LSD test, $P < 0.05$).

day and the 0700 count. High temperatures ranged from 11.4 to 14.9°C over the 3-d experiment. Low temperatures were between -7.3 and -1.4°C . The average temperature for the duration of the experiment was 3.8°C . Average relative humidity during the study was 67.7%. The data were analyzed with the same methods used in analyzing the summerform data.

Results

Summerform Test. There was a significant interaction between trial date and treatment for numbers of males trapped (Fig. 2; Table 1). Treatment effects for each trial indicated that the first trial had no significant

Table 1. Statistics from ANOVA assessing effects of treatment (traps baited with females, traps baited with males, traps unbaited) and date (17–21 July versus 28 July to 1 Aug. intervals) on capture of male or female summerform psylla and site on capture of male or female winterform psylla

Effect	df	Males		Females	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Summerform					
Date	1	14.56	0.0013	0.05	0.98
Block (date)	18				
Treatment	2	9.53	0.0005	0.19	0.827
Treatment \times date	2	3.25	0.0504	0.89	0.421
Treatment at date 1	2	0.85	0.435	—	—
Treatment at date 2	2	11.93	0.0001	—	—
Error	36				
Winterform					
Date	1	171.3	<0.0001	324.9	<0.0001
Block (site)	18				
Treatment	2	23.2	<0.0001	0.6	0.56
Treatment \times site	2	3.5	0.043	2	0.14
Treatment at site 1	2	5.9	0.006	—	—
Treatment at site 2	2	20.7	<0.0001	—	—
Error	36				

Summer form: Simple effects contrasts (treatment effects at each date separately) were extracted for male captures because of the significant treatment \times date interaction using the SLICE command in PROC MIXED. Winter form: Simple effects contrasts (treatment effects at each site (i.e., orchard) separately) were extracted for male captures because of the significant treatment \times site interaction.

differences among trap type in the numbers of males caught, whereas treatment effects were highly significant for the second trial on 28 July to 1 August (Fig. 2). For the main effects of treatment (Fig. 2C), significantly more males were caught on traps baited with females than on control traps or male-baited traps ($P < 0.05$, by LSD test). Female summerform psylla showed no preferences among the three treatments (Table 1). A total of 341 male and 352 female psylla were caught over the 4 d of the study. Eighty-nine percent of the females and 91% of the males that had been used to bait the traps survived through to the completion of the experiment.

Winterform Test. We obtained a significant interaction between treatments and sites (Table 1). At both sites, numbers of male winterform psylla on traps were affected significantly by treatment (Table 1; Fig. 3). As in the summerform psylla experiment, males were significantly ($P < 0.05$, by LSD test) more abundant on the female-baited traps than on the male-baited or control traps (Fig. 3). Trap numbers for winterform females were similar among treatments (Table 1; Fig. 3). Total numbers of psylla captured during the 6 d of the test were 1,216 females and 1,316 males. Ninety-two percent of the females and 94% of the males that had been used to bait the traps survived to the completion of the experiment.

Summerform Diel Periodicity. Summerform males were more abundant on the traps baited with females than on unbaited traps, when averaged over time of day (Table 2; Fig. 4). Trap catch also varied with time of day (Table 2). Trap catch for males showed a statistically significant increase between 0845 and 1045 hours, remained at that 1045 plateau until the 1645

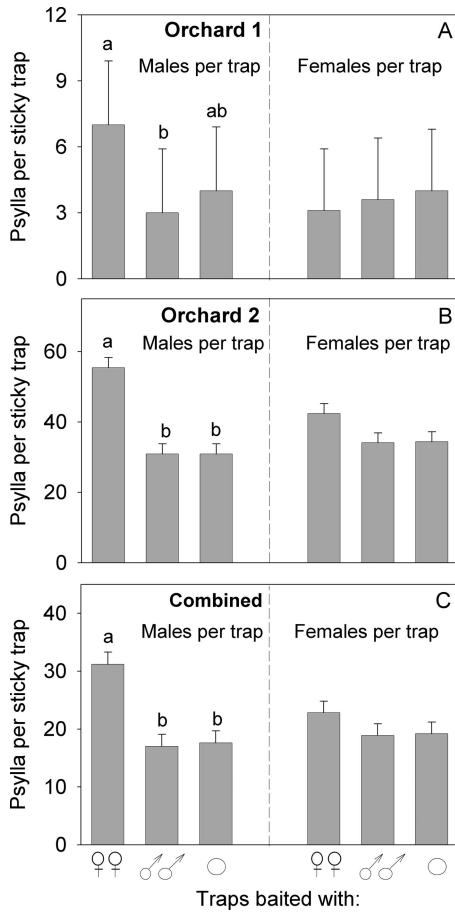


Fig. 3. (A) Mean (\pm SEM) numbers of winterform psylla caught per trap for each treatment at orchard 1. (B) Mean (\pm SEM) numbers winterform psylla caught at orchard 2. (C) Combined (\pm SEM) means from both orchards. Double male symbols on the x-axis represent traps baited with male psylla, female symbols represent traps baited with females, and the open circles indicate results for the unbaited traps. Means with different letters are significantly different (LSD test, $P < 0.05$).

sample, and showed a statistically significant decline after the 1645 sample (Table 2; Fig. 4). There was no difference between the female-baited and unbaited traps in number of female psylla caught (Fig. 4; Table 2). Profile contrasts for the time effect indicated that catch of females increased significantly after the 0845 sample and declined significantly after the 1845 sample (Table 2; Fig. 4). Total numbers of psylla captured during the 3 d of the test were 305 females and 398 males. Ninety-five percent of the females that were used to bait the traps survived to the end of the experiment.

Winterform Diel Periodicity. Winterform males were significantly more numerous on traps baited with females than on unbaited traps (Table 2; Fig. 5), but there was no significant difference between female-baited and control traps in number of female psylla caught (Fig. 5; Table 2). Trap catch for both treat-

Table 2. Statistics from ANOVA assessing effects of treatment (traps baited with females, traps unbaited) and time of day on capture of male or female summerform and winterform psylla

Effect	df	Males		Females	
		F	P	F	P
Summerform					
Time	7	10.29	<0.0001	9.3	<0.0001
0645 versus 0845 hours	1	3.64	0.058	6.06	0.015
0845 versus 1045 hours	1	18.85	<0.0001	21.34	<0.0001
1045 versus 1245 hours	1	3.21	0.074	0.49	0.484
1245 versus 1445 hours	1	0.02	0.893	0.01	0.926
1445 versus 1645 hours	1	0.08	0.773	0.61	0.435
1645 versus 1845 hours	1	5.43	0.021	3.56	0.060
1845 versus 2045 hours	1	1.69	0.194	7.26	0.008
Treatment	1	11.06	0.001	0.02	0.8941
Treatment \times time	7	0.74	0.6348	1.31	0.2447
Error	285				
Winterform					
Time	5	146.61	<0.0001	113.12	<0.0001
0700 versus 0900 hours	1	0.32	0.574	0.30	0.584
0900 versus 1100 hours	1	141.89	<0.0001	93.33	<0.0001
1100 versus 1300 hours	1	63.37	<0.0001	68.69	<0.0001
1300 versus 1500 hours	1	6.85	0.0095	11.29	0.001
1500 versus 1700 hours	1	80.28	<0.0001	55.12	<0.0001
Treatment	1	10.92	0.0011	2.1	0.1484
Treatment \times time	5	2.11	0.0659	2.12	0.064
Error	209				

Profile contrasts were extracted to compare adjacent intervals of the time factor.

ments varied with time of day (Table 2). Numbers of males and females caught on traps both increased significantly after the 0900 sample to a peak count at 1300 hours, followed thereafter by statistically significant declines in numbers (Table 2; Fig. 5). Total numbers of psylla captured during the 3 d of the test were 1,103 females and 1,269 males. Ninety-eight percent of the females that were used to bait the traps survived to the end of the experiment.

Discussion

These field trials suggested that *C. pyricola* males actively searched for females, possibly using volatiles emitted by the females, and did so during photophase (Figs. 2–5). The Asian citrus psylla, *Diaphorina citri* Kuwayama, has been observed to mate during the photophase, with matings never occurring earlier than 1 h before sunrise or later than 1 h after sunset (Weninger and Hall 2007). The diel periodicity studies done here showed that the males most readily arrived on the female-baited traps at the warmest time of the day between 1045 and 1645 hours for summerforms and between 1100 and 1700 hours for winterforms (Figs. 4 and 5). Capture of females exhibited roughly

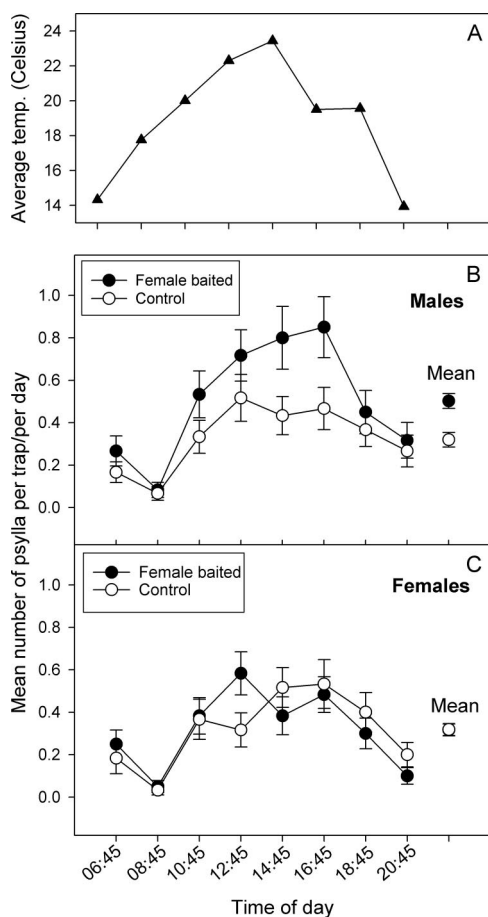


Fig. 4. (A) Temperature ($^{\circ}\text{C}$) at the end of each 2-h time block averaged over the 3 trapping d. (B) Mean ($\pm\text{SEM}$) numbers of summerform male psylla caught per trap for each trap treatment within each 2-h block, averaged over the 3-d trial. Paired symbols at far right in both panels are means of the eight time intervals. (C) Mean ($\pm\text{SEM}$) numbers of summerform females caught per trap for each trap treatment per 2-h block. Some symbol pairs may overlap. Each time interval comprised 2 h of trapping, except for the 0645 interval, which included all psylla trapped between 2045 (of the preceding day) and 0645 hours.

the same diel trend as that shown by males (Figs. 4 and 5). Both male and female psylla were consistently caught on control traps in all four experiments (Figs. 2–5), as were many other insects. Extensive capture of psylla on control traps was likely a consequence of flight activity within the orchard by both sexes during the experiments (Horton 1993).

A previous field study with *C. pyricola* used yellow sticky traps to monitor patterns of diurnal movement by males and females of both morphotypes in central Washington state (Horton 1993). That study showed that trap catch of winterform psylla increased between morning and afternoon, peaking about midday, and declining thereafter. Winterform psylla in this study showed a similar diel pattern (Fig. 5). However, patterns for summerforms in the study of Hor-

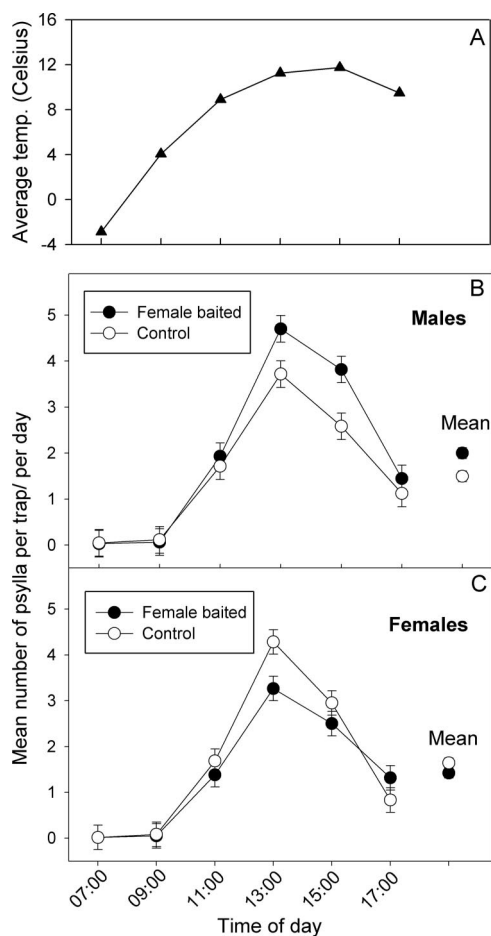


Fig. 5. (A) Temperature ($^{\circ}\text{C}$) at the end of each 2-h time block averaged over the 3 trapping d. (B) Mean ($\pm\text{SEM}$) numbers of winterform male psylla caught per trap for each trap treatment within each 2-h block, averaged over the 3-d trial. Paired symbols at far right in both panels are means of the six time intervals. (C) Mean ($\pm\text{SEM}$) numbers of winterform females caught per trap for each trap treatment per 2-h block. Some symbol pairs may overlap. Each time interval comprised 2 h of trapping, except for the 0700 interval, which included all psylla trapped between 1700 (of the preceding day) and 0700 hours.

ton (1993) are somewhat different from that observed here. Horton (1993) showed that summerforms had a slight decrease in activity at midday and that peak catch occurred between 0800 and 1200 hours. In this study, we found that summerform counts on traps increased between the morning and midday intervals, declining only as late afternoon was approached (Fig. 4). The reasons for the apparent differences between the two studies are not known. Horton (1993) did not provide temperature data, and possibly diel patterns in temperature differed enough between the two studies to affect patterns of sticky trap catch.

The literature suggests that three types of cues are used in the Psyllidae as means for locating prospective

mates: visual, acoustic (including substrate vibrations), and olfactory. For example, Krysan (1990) showed that male *C. pyricola* failed to inseminate females under conditions of total darkness in the laboratory and suggested that males may locate females at short distances using visual cues. The semiopacity of the traps used in our field experiments (Fig. 1) probably prevented males from locating females by use of visual cues, suggesting that males used either acoustic signals or olfactory detection. There is evidence that a number of other psyllid species use acoustic cues or substrate vibrations to locate or accept mates (Tishechkin 1989, 2006; Percy et al. 2006). This behavior has yet to be shown conclusively for psyllids in the genus *Cacopsylla*. Brown (2008) observed that both sexes of *C. pyricola* exhibit a wing-twitching behavior during male–female interactions, and this behavior may be evidence that insects in this psyllid species also use acoustic communication during male–female interactions (for other Psyllidae see: Ossianilsson 1950; Campbell 1964; Taylor 1985; Tishechkin 1989, 2006; Percy et al. 2006). Research to test whether *C. pyricola* uses acoustic cues in male–female sexual encounters would be of interest.

Sex pheromones occur in a number of nonpsyllid Sternorrhyncha, including mealybugs, aphids, whiteflies, and scale insects (Yin and Maschwitz 1983, Abdel-Kareim and Kozár 1988, Pickett et al. 1992, Millar et al. 2002, Walton et al. 2006). Laboratory studies have now shown that volatile cues are also used by males of some Psyllidae in locating females (Soroker et al. 2004), including by males of *C. pyricola* (Horton and Landolt 2007, Horton et al. 2007). Our field results, in combination with the earlier laboratory trials, are consistent with the hypothesis that male *C. pyricola* locate female conspecifics in the field by tracking female-emitted volatiles. Whether other cues (i.e., acoustic or visual) are also used by males in the field remains to be tested.

In summary, methods developed here provide a platform for future field tests of synthetic sex pheromones, once those pheromones have been identified and synthesized. Long-term practical benefits might include development of effective monitoring tools, use in mating disruption, or development of lure and kill technologies. Additional studies to define the environmental conditions that lead to maximal behavioral response, such as the diel trials reported here, would allow optimal use of the attractants in the field.

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